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# DIETARY FATTY ACID AND TEMPERATURE EFFECTS ON THE PRODUCTIVITY OF THE CLADOCERAN, *MOINA MACROCOPA*

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Analyses of the relationships between physical parameters and the quantity and quality of food in relation to zooplankton physiological processes such as ingestion, assimilation (growth) and reproduction have been scarce. These studies are difficult since experimental techniques have lacked means to keep biotic factors constant while modifying a physical factor, or vice versa. As a result, the nutritional quality of a particular food under certain physical conditions has not been evaluated.

The study of interactions between the quantity and quality of food available for a zooplankter and existing physical parameters is very important. The structure of zooplankton communities may be partially determined by the relative abilities of fauna to efficiently utilize and process the available food and thereby satisfy their individual nutritional requirements.

Investigators who have classified the nutritional suitability of a particular food for a predator have observed effects on ingestion, assimilation and reproduction. No study has adequately controlled experimental conditions so that the interrelationships among a physical factor, the chemical composition of diet, and the population dynamics of a zooplankton species could be identified.

Stuart, McPherson, and Cooper (1931) studied the relative value of a variety of bacterial species as food for the aseptic cladoceran, *Moina macrocopa*. They found differences in growth, fertility, and fecundity. Lefevre (1942) demonstrated that the normal growth and reproduction of various cladoceran species were dependent upon the suitability (physical or physiological) of species of fresh water algae. Monoxenic culture of two species of Crustacea, *Artemia salina,* and *Tigriopus japonicus,* an harpacticoid copepod, by Provasoli, Shiraishi and Lance (1959) demonstrated that many unialgal diets either failed to permit growth to adulthood or allowed only a few consecutive generations. Interestingly, a phytoplankton species that was nutritionally good for one species was not always good for the other species. The apparent nutritional deficiencies of some of the unialgal diets could often be rectified by the addition of specific vitamins or other organic compounds (Shiraishi and Provasoli, 1959). Lee, Tietjen, and Garrison (1976) observed a seasonal "switching" of nutritional requirements for *Nitocra typica,* an harpacticoid copepod from salt marsh aufwuchs communities. In these studies the comparative nutritional value of unialgal diets of various species and strains was determined by growth rate and fecundity measurements. In some cases the nutritional adequacy of a particular algal diet was temperature dependent. Guerin and Gaudy (1977) and Gaudy and Guerin (1977) grew the harpacticoid copepod, *Tisbe holothuriae,* on a variety of artificial chemically undefined particulate diets. The dry weight, elementary chemical composition, fecundity, sex ratio, and result-

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ing population dynamics of this species were significantly affected by the quality of food. Schindler (1971) showed that the assimilation efficiencies of the copepods *Diaptomus gracilis, Cyclops strennus,* and the cladoceran, *Daphnia longispina* are dependent upon the type of food eaten. Roman (1978) speculated that the successful association of the blue-green alga *Trichodesmium* and the harpacticoid copepod *Macrosetella gracilis* may be due to the copepod's efficient conversion of the carbon and nitrogen fixed by the alga into secondary production.

A precise understanding of how zooplankton population dynamics are influenced by the chemical composition of the diet can only be realized when proper conditions will permit the experimenter to independently control qualitative and quantitative changes. The axenic culture of a zooplankter on a chemically defined artificial diet would satisfy such controlled conditions. Such a diet must permit not only adequate ingestion, digestion, and assimilation but also continuous reproduction so that large populations and successive generations may be studied.

The biphasic medium for the axenic culture and continuous reproduction of *Moina macrocopa* formulated by Conklin and Provasoli (1978) provides for the precise study of food/zooplankton relationships. The medium consists of a soluble phase and a particulate phase. Water soluble vitamins, nucleic acids, amino acids, and inorganic salts comprise the soluble phase, while coagulated protein-starch and protein-lipid particles comprise the particulate phase. The medium has permitted the first analysis of the nutritional requirements of a crustacean (Conklin and Provasoli, 1977). By employing this medium the experimenter can control the chemical composition of the diet as well as the mode of presentation to the herbivore (*i.e.*, number of particles/ml, amount of chemical compound/particle). Since the particles, unlike natural phytoplankton and bacteria, are inert to the chemical or quantitative changes associated with physical environmental factors, precise relationships between diet composition and some physical variable can be determined.

This study is directed toward analysis of dietary fatty acid and temperature interrelationships and the effects upon the productivity of *Moina macrocopa*. Undoubtedly there are other chemical compounds which affect the normal growth and reproduction of *Moina* (Conklin and Provasoli, 1977). Experimentation with fatty acids was chosen because, for this class of compounds, there are unique compositional differences amongst the orders of algae (Wood, 1974) and because past research demonstrates the essentiality of lipid factors for sustained fertility in *Moina macrocopa* and *Daphnia magna* (Provasoli, Conklin, and D'Agostino, 1970; Viehoever and Cohen, 1938).

The importance of dietary lipids for the Crustacea was also inferred from the research on the phylogenetically related insects; lipids affect their metamorphosis, diapause, fertility, and fecundity (Beck, Lilly, and Stauffer, 1949; Vanderzant, Kerue, and Reiser, 1957; Tamaki, 1961; Dadd, 1960; Nayar, 1964; Adkisson, Bell and Wells, 1963; Bull and Adkisson, 1960; Foster and Crowder, 1976; Ikan, Stanic, Cohen and Shulov, 1970; Chumakova, 1962).

## Materials and Methods

The organism employed in this research was *Moina macrocopa americana,* a member of the order Cladocera. Descriptions and illustrations may be found in

Goulden's monograph of the Moinidae (Goulden, 1968). Reproduction occurs primarily by parthenogenesis. At times sexual females and males will appear in populations and sexual reproduction will occur (D'Abramo, in preparation). Parthenogenetic eggs are released from a pair of ovaries into a brood pouch. The neonates are born viviparously and are miniature images of the adults. Neonates pass through four stages of growth (instars) before becoming sexually mature. During the fourth or adult instar a female lays her first brood of eggs. Generation time is temperature-dependent but is rapid compared to many other Crustacea (4–5 days at 26° C).

*Axenization*

A serial dilution method as described by Conklin and Provasoli (1978) was employed to free the experimental organisms from bacteria. Recently born neonates were washed for 15 min in 15 separate baths composed of 10 ml of $DM_7$ medium (Provasoli and D'Agostino, 1970) in 6-cm diameter Petri dishes. All baths contained two drops of an antibiotic mix (D'Agostino and Provasoli, 1970). The eighth bath of the series contained a suspension of bacteria-free *Chlamydomonas reinhardii* ($GMS^-$), to allow for feeding and clearing the gut of bacterial flora. After the entire washing procedure, individual animals were transferred to 20 × 125 mm screwcap test tubes containing 10 ml of $DM_7$ and a suspension of *C. reinhardii*. After two days growth at room temperature under a bank of cool white fluorescent bulbs, 0.5 ml of the culture medium was dispensed into semisolid DA medium (D'Agostino and Provasoli, 1970) for detection of any bacterial or fungal contaminants. This sterility test was incubated at room temperature for 15 to 20 days.

Once monoxenic cultures were established and found aseptic, young were aseptically transferred to 20 × 125 mm screwcap test tubes that contained 10 ml of a sterile artificial medium, $K_733$, which is a modification of the artificial medium developed for *Moina* by Conklin and Provasoli (1977) (Table I). Acclimation to the artificial medium occurred within three generations and was determined by visual observation of the number of progeny per brood and of a normal swimming behavior. Culturing of animals fed artificial food was done in the darkness at room temperature. Growth in the dark discourages algal reproduction and all traces of algal cells generally disappeared by the third transfer in artificial media.

Populations of *Moina* were maintained on artificial and natural diets. Maintenance populations on artificial medium $K_733$ were incubated at 19° C in darkness to prevent photodeterioration of riboflavin. Growth at this temperature slowed population growth rates, thereby increasing the time between transfers. Populations fed *Scenedesmus naegalii* (Chodat) and *C. reinhardii* ($GMS^-$) were grown at 19° C with a 16:8 L/D photoperiod. New cultures of natural and artificial diets were usually started every three weeks.

*Diet formulation*

The artificial media used in the experimentation with *Moina macrocopa* were modifications of the F1 medium of Conklin and Provasoli (1977). Modifications may be found in Table I. The media contained two types of particles, starch-

TABLE I

*Modifications of Conklin-Provasoli F1 Medium.*

| | | |
|---|---|---|
| Common basal medium (per cent w or v/v) | | |
| Changes: | (a) | Metal mix P II to metal mix L; Metal mix L, 1 ml = $Na_2EDTA \cdot 2H_2O$, 3.81 g; Zn(as $SO_4^=$), 0.30 mg; B, 0.12 mg; Mn(as $Cl^-$), 0.087 mg; Fe(as $NH_4SO_4^-$), 0.06 mg; Co(as $Cl^-$), 0.024 mg; Cu(as $SO_4^=$), 0.024 mg; Mo(as $NH_4^+$), 0.036 mg. |
| | (b) | Liver infusion L25 at 70 mg to defatted liver infusion L25 (lipid extraction by chloroform/methanol (2/1) (v/v) for three hr under $N_2$) at 40 mg. |
| Additions: | (a) | glycogen 10 mg |
| | (b) | globulin + 2X crystalline egg albumin, 1 ml, 1 ml = bovine $\alpha$ globulin (Fraction IV) 2.25 mg + 2X crystalline egg albumin (ICN), 0.75 mg. Mixture is formed by dissolving components in water; coagulating this mix by autoclaving; homogenizing (2600 rpm for 5 min) the coagulum to produce particles; autoclaving the particles and rehomogenizing. |
| Deletions: | (a) | $DF_2$ |
| Particles | | |
| Changes: | (a) | SA gel particles: 1 ml = 10 mg rice starch + 4.10 mg 2X crystalline egg albumin. |
| | (b) | FV particles: 1 ml = 8 mg 2X crystalline egg albumin + 0.75 egg lecithin + 1 mg BHT (butylated hydroxytoluene) + 0.66 ergocalciferol + 0.25 retinopalmitate + 2 mg dl-$\alpha$tocopherol + fatty acids in variable quantities and qualities, depending upon experiment. Fatty acids for $K_733$ medium: 1 mg palmitic acid + 0.3 mg oleic acid + 0.7 mg linoleic acid + 1 mg $\alpha$-linolenic acid. |
| Artificial medium | | |
| Basal medium 96 ml + 3 ml SA gel + 1 ml FV particles, pH = 8.0, Particle concentration = $540 \times 10^3$ ml | | |

protein and lipid-protein. All inorganic and organic additions were made from prepared stock solutions. Generally inorganic stock solutions were stored at room temperature while organic solutions or suspensions were stored either at 8° C or frozen. To prevent bacterial contamination, 0.5 ml of a volatile preservative solution (Hutner and Bjerknes, 1948) was added to all stock solutions. The volatile preservative vaporizes during autoclaving. Stock solutions were usually renewed within a four-month period. All fatty acids used in the diets were 99+% pure and were stored frozen under a $N_2$ atmosphere. Prepared media varied in the qualitative and quantitative composition of fatty acids absorbed onto the protein particles. Qualitative fatty acid additions were made to simulate the unique proportional differences found amongst four orders of algae, Cyanophyceae, Cryptophyceae, Chlorophyceae, and Bacillarophyceae (Table II).

Since the work involved the effect of quantitative and qualitative changes in dietary fatty acids upon the productivity of *Moina*, concern developed regarding the possible differential uptake of particular fatty acids by the albumin. To insure that results could be genuinely attributed to particular diets, particles prepared 2 months previously were subjected to a lipid compositional analysis by thin layer and gas chromatographic techniques. These analyses were kindly performed by Dr. David H. Beach of the Department of Microbiology, State University New York, Upstate Medical Center at Syracuse. Lipid analysis of the particles revealed

TABLE II

*Fatty acid mixtures simulating average percent composition of representative algal classes (total fatty acids = 3 mg).*

| Fatty acids (mg) | Mixtures | | | |
|---|---|---|---|---|
| | Cyanophyceae[1] | Chlorophyceae | Cryptophyceae | Bacillariophyceae |
| 14:0 (myristic) | — | — | 0.30 | — |
| 16:0 (palmitic) | 1.25 | 1.00 | 0.45 | 0.75 |
| 16:1 (palmitoleic) | 0.80 | 0.10 | — | 1.20 |
| 18:0 (stearic) | 0.12 | — | — | — |
| 18:1ω9 (oleic) | 0.30 | 0.25 | 0.30 | 0.15 |
| 18:2ω6 (linoleic) | 0.40 | 0.35 | 0.30 | — |
| 18:3ω3 (α-linolenic) | 0.13 | 1.30 | 0.90 | — |
| 20:5ω3 (eicosapentaenoic) | — | — | 0.45 | 0.90 |
| 22:6ω3 (docosahexaenoic) | — | — | 0.30 | — |

[1] The 18:4 (octadecatetraenoic acid) which comprises from 15 to 30% of the cryptophyceae fatty acids was not incorporated into the mixture because of the lack of a conveniently available and highly pure (99 + %) source.

that the experimentally intended dietary differences, both qualitative and quantitative, were genuine. The lipophilic albumin had no tendencies toward differential absorption of the fats and vitamins. Uptake was complete.

*Determination of particle size and particle concentration—optical transmission relationships*

The number of particles in media of different particle concentrations was determined through the use of a Coulter Counter (Model $Z_B$, Counter Electronics, Hialeah, Florida). The counter was calibrated with the use of 10.2-μ diameter pollen. Counts of artificial particles were performed using a 100-μ aperture, an amplification of four and an aperture current equal to one half. The lower and upper threshold settings were 1 and 40, respectively. The threshold factor measured as the average volume of the known system divided by the lower threshold dial setting at half count equalled 16.8186. Particle size frequency distribution for a sample was determined using a Coulter Channelyzer (base channel threshold = 1, window width = 100) and an X-Y Recorder II for automatic plotting. The size of the particles was determined by employing the formula, Channel number × window width/100 + Base Channel Threshold × Threshold Factor = cubic microns.

Aliquots of variable volumes from samples of percent transmissions ranging from 45 to 85% were diluted to 20 ml by means of a special electrolyte solution, Isoton II (Curtin Matheson Scientific, Inc.). From these diluted suspensions ten separate 500-μl samples were counted, from which an average was computed. Counts for these samples ranged from 10,000 to 30,000 per 500 μl. Background counts did not exceed 100 per 500 μl. The derived average particle concentration (number per 500 μl) was then multiplied by the dilution factors to obtain the number of particles per ml. A standard curve relating particle concentration (number/ml) to per cent transmission was then constructed. Particle concentration within the media was altered by additions from the stock mixtures. All prepared media were

adjusted to pH 8.0 with a pH meter and were then dispensed with a macropipette (Macroset-Oxford Laboratories) as 10 ml aliquots into 20 × 125-mm screw cap culture tubes (Pyrex # 9825). The media in the culture tubes were then autoclaved, and, after cooling to room temperature, were stored at 8° C until use. Experiments involving the comparative quality of diets were all performed with media containing the same initial number of particles. Normal concentration was $540 \times 10^3$ particles/ml.

### *Preparation of inoculum and productivity determinations*

To determine the relative nutritive quality of diets with each particular fatty acid composition, 10 to 12 first instar females were inoculated into separate culture tubes containing the same variable. These inocula were neonates of the first and second broods of single females that had been previously isolated from maintenance cultures and grown at 19° C in 20 × 125-mm screw cap culture tubes containing 10 ml of $K_7$33 media. From these, replicate inocula populations were allowed to develop by parthenogenesis at various temperatures and defined times. The particles of the artificial media were kept in suspension by daily mixing with a Vortex-genie mixer (Scientific Industries, Inc.). Particle sedimentation rates were slow and most of the particles (*ca* 70%) would remain in suspension over a 24-hr period. From the 10 to 12 growing populations a set of three or four culture tubes were harvested at three different predetermined particle concentrations (60–70%, 70–80%, 80%+ optical transmission at 650 nm). Optical transmission was measured by an instrument similar to a Spectronic 20 (Bausch and Lomb) but modified to accept culture tubes.

The sequential harvesting at different particle concentrations permitted an analysis of the changing structure of the *Moina* populations through time. Animals were killed by the addition of 0.5 ml of ethyl alcohol and then were transferred by pipette to a modified Bogorov counting tray (Wickstead, 1965). With the aid of a binocular stereoscope (36×) each individual comprising a population was counted, sized, and categorized. Categories included female instar I-IV, male instar I-IV, adult females, adult males, ephippial females, and ephippia. Measurements of the animals were made with the use of an ocular micrometer. Measurements were made anteroposteriorly, from a point just distal to the eye to the caudal tip of the carapace.

Average dry weights of the four female instars were determined by selecting a sufficient number of animals of a particular instar and placing them on a pretared aluminum dish. These samples were then dried at 60° C for 24 hr in a laboratory oven (Model 10–200C, Grieve-Hendry, Co., Chicago, Ill.), cooled for one hr in a desiccator, and weighed immediately on a Cahn gram electrobalance (Model G-Cahn Instrument Co., Paramount, California). Additional weighings of a sample were performed until no change in weight could be detected. Average dry weight of a particular instar was determined by dividing the biomass in the dish by the number of instars which comprised the sample. Three samples of well-fed animals were taken for the average weight determination of each instar. Each sample was derived from a population growing on a different diet. A $\log_{10}$-$\log_{10}$ plot of average length of an instar *vs.* average dry weight indicated that

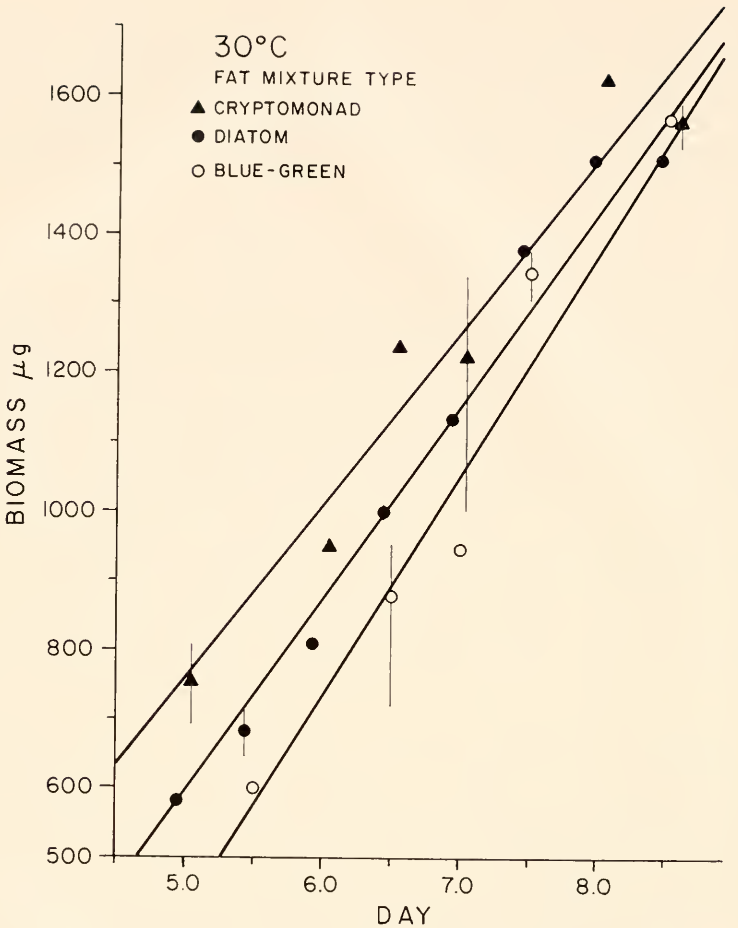


FIGURE 1. Fatty acid composition dependent qualitative effect. 30° C; diets containing cryptomonad, diatom, and blue green fatty acids at 3 mg%.

the dry weight of *Moina* increases as the cube of the body length. The equation is: dry weight = 1.1 body length$^3$; corr. = 0.9992. The total biomass of a population of *Moina* was then determined by converting lengths of each individual comprising the population into dry weight and summing the total.

Productivity was determined by dividing the total biomass of an harvested population by the total number of days since the appearance of the first brood of the original female inoculum. The day when the inoculum's first brood appeared was considered day one in order to eliminate some of the observed individual variability associated with time to maturity and thereby provide comparable data among samples of the same medium.

Many of the populations analyzed contained males and ephippial females but their presence did not affect productivity calculations since they invariably had not attained sexual maturity when the populations were harvested.

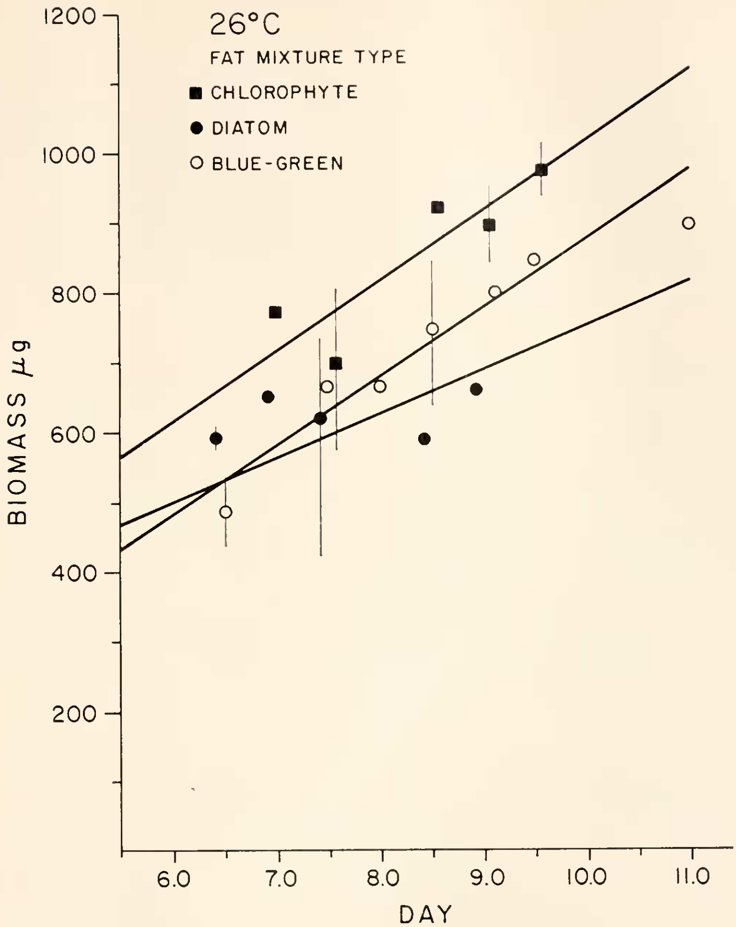


FIGURE 2. Fatty acid composition dependent quantitative effect. 26° C; diets containing chlorophyte and blue green fatty acids at 3 mg% and diatom fatty acids at 1.5 mg%.

All experimental populations were incubated in darkness. This procedure was used to eliminate the photoperiod factor which has been associated with the transition to sexuality in some species of Cladocera and to prevent the photodegradation of riboflavin, a required vitamin present in all media. Exposure to light was minimal and occurred only when cultures were shaken or when optical transmission measurements were taken. The source of light was indirect, consisting of a 10 watt light bulb fitted into a photographic safelight stand which was covered with red acetate film. Within the optical transmission apparatus red acetate material covered the slit (passage) between the light source and the area where the culture tube was positioned for a measurement. Light transmitted by the red acetate material was 600 to 700 nanometers. Past research indicates that Cladocera are minimally responsive to wavelengths in this region in both visual and nonvisual sensitivity (Scheffer, Robert, and Medioni, 1958).

## Results

### *Particles*

Two types of particles were used as food in all media. It was important, therefore, to determine their size distribution. Although formulated separately, starch-protein and lipid-protein particles did not differ in size distribution. Particle size ranged from 2 to 20 $\mu^3$ with the majority of particles (80%) in the 2 to 10 $\mu^3$ range. Because of the similar size distributions it was assumed that there was no different sedimentation rate which could affect the probabilities of ingestion of the two types of particles.

### *Effects of dietary fatty acids and temperature on productivity*

A series of experiments was done to define the effect of varying the quantity and quality of dietary fatty acids at different temperatures. Results of the experiments are shown in Figures 1, 2, and 3. Points of graphs represent either a single determination or an arithmetic average of multiple (2–4) determinations of biomass of populations developed on particular diets at selected days of harvest. Points derived from multiple observations include the range of values (vertical bars). Computed regression lines are included. Mortality within populations did not affect productivity determinations since, in most cases, the maximum length of an experimental period never exceeds the life span of *Moina*. If mortality was observed the population was discarded.

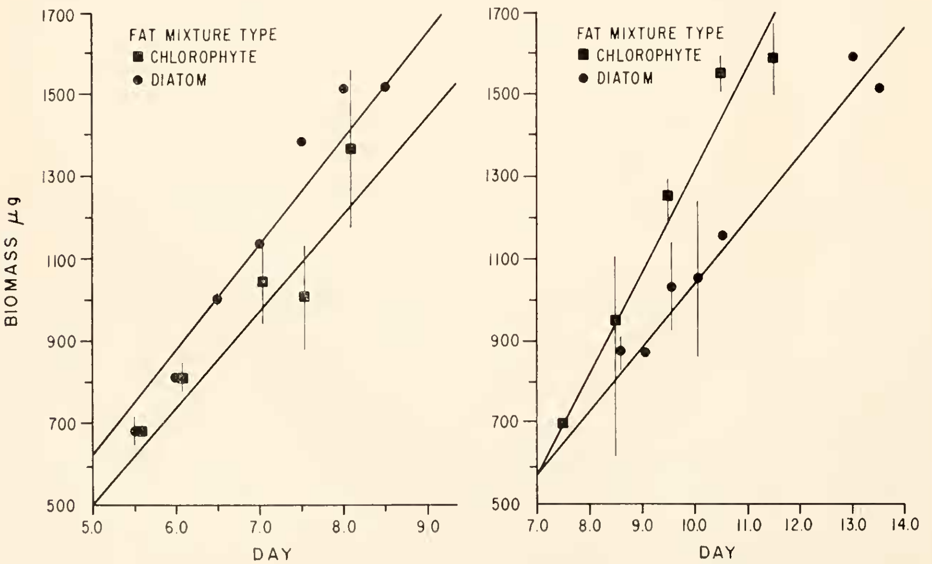


FIGURE 3. Fatty acid composition dependent temperature effect. Diets containing chlorophyte and diatom fatty acids at 3 mg%, 30° C and 22° C.

TABLE III

*Productivity ($\mu g$/day, $\bar{x} \pm$ s.d.)—temperature relationships; diets containing green or cryptomonad fatty acids at 3 mg%. Initial particle concentration = $540 \times 10^3$/ml, (N) = number of observations.*

| Diet | Fatty acid concentration | Temperature | | | | |
|---|---|---|---|---|---|---|
| | | 30° C | 26° C | 22° C | 18° C | 14° C |
| Cryptomonad | 3 mg% | 173.4 ± 22.4 (11) | 163.4 ± 31.1 (13) | 122.4 ± 32.5 (10) | 58.0 ± 7.1 (12) | 49.2 ± 8.0 (12) |
| Blue green | 3 mg% | 155.0 ± 31.6 (11) | 151.3 ± 27.3 (10) | 108.4 ± 19.6 (12) | 51.4 ± 5.5 (11) | 44.5 ± 7.0 (12) |

TABLE IV

*The relationship of productivity ($\mu g$/day, $\bar{x} \pm$ s.d.) and concentration of dietary fatty acids. Diets containing chlorophyte fatty acids, 3 and 6 mg% at 22° C. Diets containing cryptomonad fatty acids at 1.5 mg%, 3.0 mg% and 6 mg% at 26°, 22°, and 14° C. Initial particle concentration = $540 \times 10^3$/ml, (N) = number of observations.*

| Diet | Fatty acid concentration | Temperature | | |
|---|---|---|---|---|
| | | 26° C | 22° C | 14° C |
| Cryptomonad | 1.5 mg% | 89.2 ± 12.7 (13) | 89.4 ± 15.7 (11) | 36.0 ± 7.7 (13) |
| Cryptomonad | 3.0 mg% | 163.4 ± 31.2 (13) | 122.4 ± 32.5 (10) | 49.2 ± 8.0 (12) |
| Cryptomonad | 6.0 mg% | 127.0 ± 14.7 (11) | 93.1 ± 10.3 (10) | 35.8 ± 6.4 (11) |
| Chlorophyte | 3.0 mg% | | 125.1 ± 20.1 (11) | |
| Chlorophyte | 6.0 mg% | | 113.4 ± 14.0 (13) | |

Figure 1 illustrates the qualitative effect of dietary fatty acid composition. At 30° C and 3 mg% total fatty acids, a diet containing cryptomonad-type fatty acids is more productive than those of diatom and blue green type fatty acids. Figure 2 depicts the quantitative effect of dietary fatty acid composition. In this experiment the three diets were supplied at one-half the normal total particle concentration and the content of total fatty acids per particle was varied. At 26° C the diatom type fatty acid diet containing 1.5 mg% of fatty acids is as productive as the blue green type diet which contains 3.0 mg% fatty acid. The chlorophyte fatty acid diet at 3.0 mg% fatty acid concentration is more productive than both. Hence at 26° C the qualitatively poor blue green diet barely equalled the productivity of the qualitatively better diatom diet even with twice the total calorific content.

Figure 3 exemplifies the effect of temperature in relation to the quality of dietary fatty acids. At 30° C and 3 mg% fatty acid concentration, the diet containing diatom fatty acids is slightly more productive than the diet containing the chlorophyte fatty acids. This relationship is inversed at 22° C.

One way ANOVA indicates that productivity differences between chlorophyte (3 mg%) and diatom (1.5 mg%) fatty acid diets at 26° C, the chlorophyte (3 mg%) and blue green (3 mg%) fatty acid diets at 26° C and the chlorophyte (3 mg%) and diatom (3 mg%) fatty acid diets at 22° C are significant ($P < 0.05$).

The productivity of every diet decreased as the incubation temperature decreased. This relationship is illustrated in Table II where productivity values of a cryptomonad fatty acid diet at 3 mg% fatty acid and a blue green fatty acid diet at 3 mg% fatty acids are listed at five different temperatures, 30°, 26°, 22°, 18°, and 14° C. One way ANOVA indicates that differences between these diets at 22° and 18° C are significant ($P < 0.05$).

Increasing the total amount of fatty acids per particle in diets while keeping the starch-protein ratio constant at 1.5 : 1 had an effect on productivity at the three different temperatures studied. Three diets were made to contain cryptomonad-type fatty acids in concentrations of 1.5, 3.0 and 6.0 mg% respectively. Table III shows that at those temperatures investigated, 22°, 18°, and 14° C, productivity increased from 1.5 to 3.0 mg%. However, an increase to 6.0 mg% reduced productivity, indicating that a too high dietary fat content is inhibitory. An increase in total fatty acids from 3.0 to 6.0 mg% in the chlorophyte type fatty acid diet was also inhibitory at 22° C.

## Discussion

Axenic culture of *Moina macrocopa* in almost chemically defined biphasic media has allowed a precise analysis of some dietary factors affecting its population dynamics, and particularly a better understanding of the combined effects of abiotic and biotic factors. It has been established that the chemical composition of food, particularly the type and content of fatty acids, can directly affect productivity. Qualitative fatty acid differences in the diets were formulated in an attempt to simulate the general composition that is unique to each of four orders of algae, Cyanophyceae, Chlorophyceae, Bacillarophyceae, and Cryptophyceae. Variations

in productivity amongst the diets can most probably be attributed to the combined effects of differences in generation time, brood size, and time between broods. The comparative quality of a particular diet can also be temperature dependent.

The poor quality, in general, of those diets containing Cyanophyceae-type fatty acids may partially explain the observed poor nutritional value of some species of blue-green algae (Arnold, 1971). A partial explanation of the different nutritiousness of various species of algae for zooplankton species may in fact be the qualitative and quantitative content of dietary fatty acid. Though no fatty acid analyses were performed on *Moina* organisms grown on particular diets, the notable differences in productivity of the various diets suggest that body fatty acids are derived entirely from dietary sources. The qualitative fatty acid composition of the lipids of *Moina* has been found to be significantly affected by diet (Watanabe, Arakawa, Kitajima, Fukusho, and Fujita, 1978). It appears that the zooplankter's capacity for efficient lipid biosynthesis or inter-conversion is poor. Fatty acids were found to be essential to fertility in *Moina* (Conklin and Provasoli, 1977).

The results of the research conducted with increased fatty acid quantities (1.5 mg%-3 mg%-6 mg%) suggest that subtle interrelationships can exist amongst macronutrient dietary components. Little or no increase in the productivity of *Moina* was attained by increasing the total cryptomonad fatty acids concentration from 3 to 6 mg% at the different temperatures. The same results occurred for a chlorophyte type fatty acid diet at 22° C. Protein and carbohydrate concentrations remained constant in these experiments. Such observations add to the convincing evidence that crustaceans cannot tolerate high levels of dietary lipid. Andrews, Sick, and Baptist (1972) demonstrated that a dietary lipid supplement (1/3 beef tallow, 1/3 corn oil, and 1/3 menhaden oil) at levels >10% adversely affected growth and survival in the shrimp *Penaeus setiferus*. Forster and Beard (1973) supplemented a shrimp meal based diet for *Palaemon serratus* with 7.5 and 15% levels of cod liver and corn oil. At the 15% level significant growth inhibition was observed for both lipid sources. The inhibitory effects of increased fatty acids in the diet may also partially explain the nutritional inadequacy of senescent algal cells. Twenty-five percent of the total dry weight of these cells is known to be fat (Fogg, 1965).

Optimal productivity of *Moina macrocopa* requires the presence of macronutrients in the proper proportions. Provasoli and D'Agostino (1969) showed that optimal starch-protein ratios for the growth of *Artemia salina* were 5 : 1 and 10 : 1. A 1 : 1 ratio was inhibitory. Hence, the potential quality of food sources cannot be entirely evaluated from considerations of calorific content.

The interesting physiological responses of *Moina* to varied nutrition permit some speculation. It seems plausible to assume that given the same food, the growth and reproductive capacities of species of zooplankton can be entirely different. Zooplankton community structure in an aquatic system may in part be determined by competitive processes whose outcome is based upon the satisfaction of nutritional requirements and/or the most efficient systems of biochemical assimilation and conversion.

The subtle nutritional interrelationships between predator and prey must be

identified if successful continuous culture of other phagotrophic invertebrates like *Moina* is to be realized. With a proper understanding of nutritional requirements and efficient energy budgets, the potential husbandry of marine particulate feeders such as lobsters, oysters, scallops, and shrimp can be greatly enhanced. Knowledge of crustacean nutrition may permit the use of cladoceran cultures as an alternative secondary sewage treatment. Filter feeding of particulates by the Cladocera would eliminate much of the potential biological oxygen demand (BOD) of primary treatment effluent and resulting populations could be harvested and used as fish food.

This work represents part of a dissertation submitted to the Graduate School of Yale University in partial fulfillment of the requirements for the Ph.D. degree, granted in May, 1979. I am deeply grateful to Dr. Luigi Provasoli for his advice and encouragement. This research was supported by a Sigma Xi grant and NSF grant DEB77–07226 to the author and by NSF grant DEB77–05433 to L. Provasoli.

## Summary

1. The cladoceran, *Moina macrocopa americana* was cultured axenically on an artificial diet consisting of a particulate and soluble phase. The effect of changes in the quantitative and qualitative dietary fatty acid composition was investigated.

2. Qualitative fatty acid differences were made to simulate the unique proportional differences found among four orders of algae, Cyanophyceae, Chlorophyceae, Cryptophyceae, Bacillarophyceae.

3. The quality of dietary fatty acids available to *Moina* exerts an effect upon productivity and the nutritional value of a particular diet in relation to fatty acid composition can be temperature dependent.

4. Increased levels of fatty acids in the diet of *Moina* reduces productivity.

5. The cladoceran, *Moina macrocopa americana,* may be entirely dependent upon diet for its source of fatty acids.

6. A partial explanation for the differential nutritiousness of particular species of algae may be their qualitative and quantitative fatty acid content.

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